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TITLE: Regulation and Action of SKP2 in Cell and Tumor Models: Mechanisms Underlying Aggressive Growth in Basal-Like Breast Cancer

PRINCIPAL INVESTIGATOR: Katerina Fagan-Solis, B.S.

CONTRACTING ORGANIZATION: University of Massachusetts
Amherst, MA 01003-9333

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The objective of this research is to further our understanding of the molecular mechanisms underlying the aggressive growth of estrogen receptor (ER)-negative, basal-like breast tumors. My goal is to determine if SKP2 is a viable new therapeutic target to specifically treat patients who have tumors that are independent of ER signaling. The most significant result was determining that knockdown of SKP2 in TMX2-28 cells shifted the cell cycle resulting in a significant increase in the percentage of cells in the G1/G0 phase, as well as a significant decrease in the percentage of cells in the S-phase of the cell cycle.

15. SUBJECT TERMS
ER-negative, Breast Cancer, SKP2

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Table of Contents

Introduction:

The objective of this research is to further our understanding of the cellular and molecular mechanisms underlying the aggressive growth of ER-negative, basal-like tumors. The goal is to identify new therapeutic targets to specifically treat patients that have tumors that are independent of ER signaling as these tumors are more often ER-negative. Past work from our lab and others has suggested that S-phase kinase-associated protein 2 (SKP2) plays an important role in breast tumorigenesis and would make a good therapeutic target. By utilizing three models (human tissue, animal models, and tissue culture) in which to characterize the role of SKP2 in breast cancer, we can obtain a better understanding of the molecular mechanisms underlying the aggressive tumor growth of basal-like breast tumors. It is anticipated that results from these studies will show that SKP2 would make a good therapeutic target for the treatment of women with basal-like tumors that are often associated with poor clinical outcome and tend to be ER-negative.

Body:

Task 1: I have received 25 ER-negative FFPE tissue cases, 18 of which are triple negative, from the Cancer Tissue Bank at UMASS Worcester, and will be receiving ~70 additional cases from their archives. These tissues have been stained for SKP2 and its associated proteins and are currently being scored in collaboration with Dr. Otis at Baystate Medical Center.

Task 2: I have successfully created a mixed population, as well as a number of single clone populations of TMX2-28 cells that has been stably transfected with the negative control SKP2-shRNA vector. Additionally, I have successfully created a mass culture population of TMX2-28 cells that have been stably transfected with SKP2-shRNA vector. Single clone populations of the SKP2-shRNA transfected cell line have been established.

Task 3: Alterations in cell cycle have been studied in the single clone population of SKP2 knockdown TMX2-28 cells compared to negative control-shRNA transfected cells. Cell cycle analyses of a mass culture of population of knockdown cells were also studied to confirm knockdown effects.

Task 4: *In vivo* studies of cell proliferation upon knockdown of SKP2 have begun.

Task 5: Preparations of dissertation and publication manuscripts have begun.

Key Research Accomplishments:

Training Accomplishments:

- Continue collaborations with **Dr. Christopher Otis**, Director of Surgical Pathology at Baystate Medical Center; **Dr. Brian Pentecost**, New York Department of Health,; **Dr. Sallie Smith-Schneider**, Pioneer Valley Life Sciences Institute; and **Dr. Douglas Anderton**, Associate Dean for Research Affairs, Director of Social and Demographic Research Institute
- Current and active member of AACR, AAAS, and SACNAS
- Continue to talk and meet with my mentor Dr. Kathleen Arcaro on a daily basis
- Attend weekly cancer and chemoprevention journal club, apoptosis journal club, molecular and cellular biology seminar and colloquia, animal biotechnology and biomedical science seminar
- Attended and presented research at a number of cancer research conferences

Research accomplishments:

- Obtained 25 ER-negative FFPE tissue cases, and will obtain ~70 additional cases in order to evaluate SKP2 and its associated protein’s expression.
- Continued pathological studies of SKP2 pathway protein in human breast cancer samples

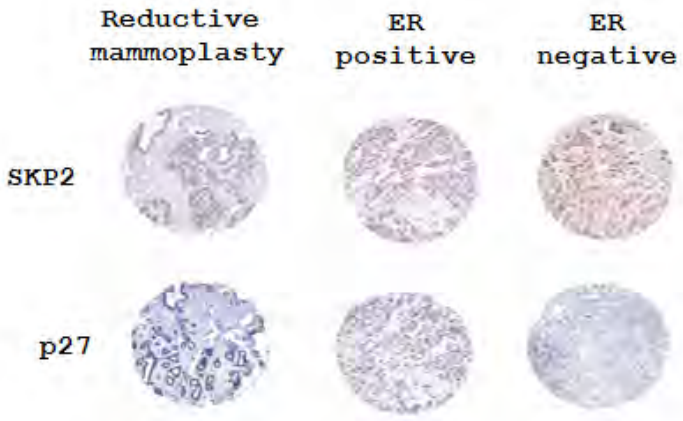
- Determined alterations in cell cycle resulting from SKP2 knockdown in TMX2-28 cells
- Began *in vivo* studies on growth and metastasis using TMX2-28 cells as well as SKP2 knockdown TMX2-28 cells

Reportable Outcomes:

To study the role of SKP2 in triple-negative and basal-like breast cancer, the tamoxifen-selected breast cancer cell line, TMX2-28, was used as a model for aggressive growth and invasion. TMX2-28 cells are triple-negative with a basal cytokeratin expression pattern. In contrast to the ER-positive, parent cell line, MCF-7, TMX2-28 cells display aggressive growth and increased invasiveness as evidenced by a reduced doubling time, prolonged S-phase, and invasion through a transwell assay.

We found SKP2 to be overexpressed in 7 out of 30 frozen breast carcinoma samples and to be higher in tumors that were ER-negative and expressed basal cytokeratins 5 and/or 17. Moreover, I found SKP2 to be highly expressed in 46% of ER-negative tumors, 24% of ER-positive tumors, and 18% of reduction mammoplasty tissues. Importantly, SKP2 was highly expressed in 77% of triple negative breast cancers. Currently, I am analyzing additional triple-negative breast cancer tissues in order to further delineate these findings.

SKP2 PROTEIN EXPRESSION IS HIGH IN 60% OF ER-NEGATIVE BREAST CANCERS



	SKP2 positive	SKP2 negative	
ER-negative	26	78	104
ER-positive	21	14	35
Reductive mammoplasty	5	45	50
	52	137	

Figure 1: SKP2 expression was examined in 35 ER-negative, 104 ER-positive, and 50 reductive mammoplasty tissue samples by immunohistochemisty. SKP2 protein was found to be highly expressed in 60% (21 of 35) of ER-negative tumors and 25% (26 of 104) of ER-positive tumors, and 10% (5 of 50) reductive mammoplasty tissues. Additionally, 44% (8 of 18) of ER-negative tumors expressed high SKP2 and low p27. Representative SKP2 and p27 stained punches are shown.

I determined that SKP2 mRNA and protein are overexpressed in TMX2-28. Additionally, TMX2-28 cells overexpress a number of cell cycle genes associated with SKP2, including p27, CDK2, and cyclin E. Transient knockdown of SKP2 expression did not significantly alter gene expression of the associated genes.

TMX2-28 CELLS OVEREXPRESS A NUMBER OF CELL CYCLE GENES ASSOCIATED WITH SKP2

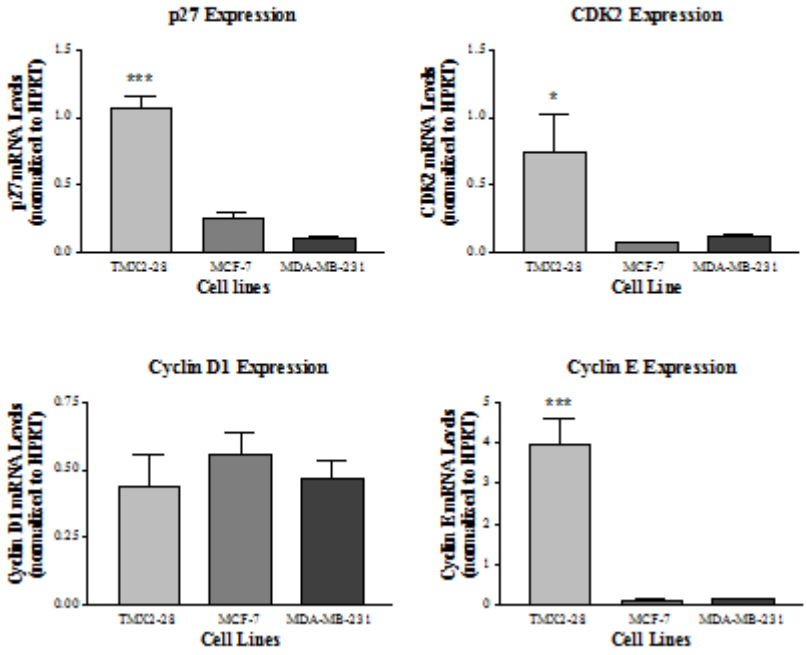


Figure 2: Gene expression was determined using real time qRT-PCR (One-way ANOVA, p27: p=0.0326; CDK2: p<0.0001; Cyclin E: p<0.0001)

KNOCKDOWN OF SKP2 IN TMX2-28 CELLS DOES NOT RESULT IN SIGNIFICANT CHANGES IN THE GENE EXPRESSION OF THE CELL CYCLE GENES ASSOCIATED WITH SKP2

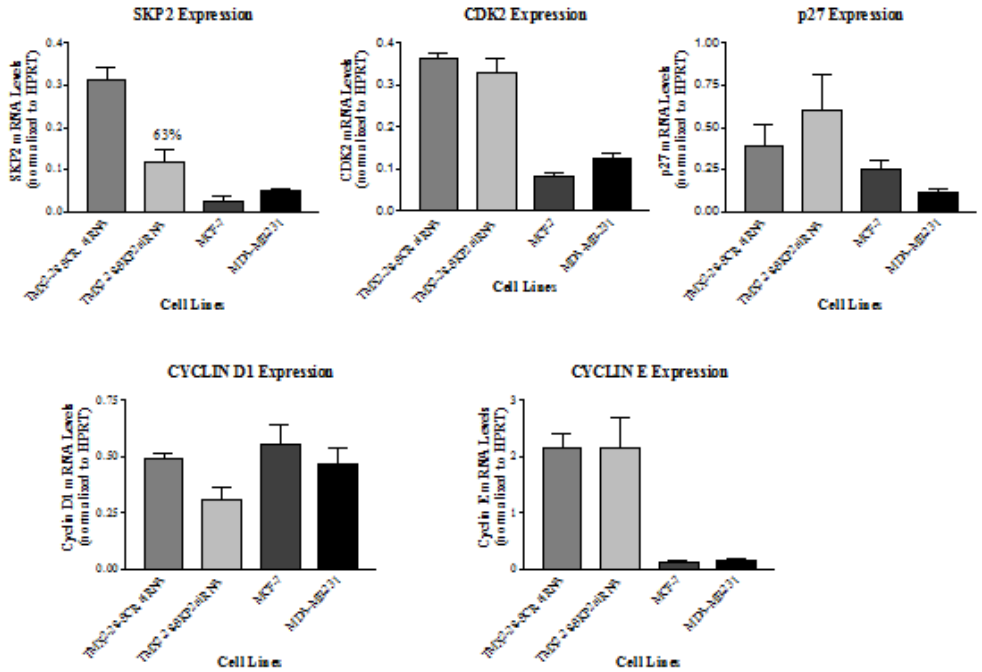


Figure 3: TMX2-28 cells were transiently transfected with siRNA targeting SKP2 or a scrambled (SCR.) version of the sequence (negative control) using a lipid based transfection agent. Forty-eight hours post transfection RNA was isolated and gene expression was determined using real time qRT-PCR (Unpaired T Test with Welch's correction)

Knockdown of SKP2 in TMX2-28 cells shifted the cell cycle resulting in a significant increase in the percentage of cells in the G1/G0 phase, as well as a significant decrease in the percentage of cells in the S-phase of the cell cycle. However, there was not a significant difference in the percentage of cells in the G2/M phase of the cell cycle. I

am currently using these stable knockdown cell lines for in vivo studies of tumor growth and metastasis

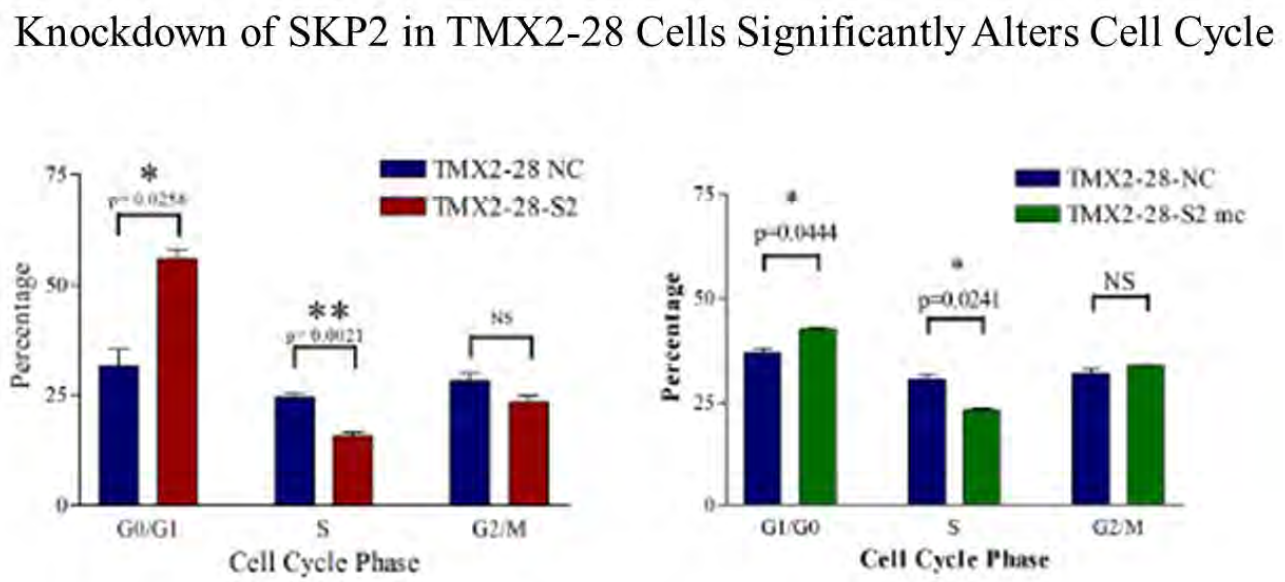


Figure 4: As predicted, knockdown of SKP2 in TMX2-28 cells resulted in a significant increase in the percentage of cells in the G1/G0 phase, as well as a significant decrease in the percentage of cells in the S-phase of the cell cycle. Contrary to our prediction, there was not a significant difference in the percentage of cells in the G2/M phase of the cell cycle. These results were consistent between experiments as well as between the single clone and mass culture knockdown cells.

Conclusion:

Triple-negative and basal-like breast cancer continues to pose a major challenge to clinicians. Given that triple-negative and basal-like breast cancer patients are without targeted therapies, clinicians are left to relay on non-specific, cytotoxic agents. To develop targeted therapies, the approach must be geared towards the molecular biology of the tumor. Additionally, development of predictive markers can optimize the success of therapeutics. Overexpression of SKP2 can serve as a predictive marker for women at risk for aggressive tumor growth. SKP2 provides a potential target for therapeutics in which triple-negative and basal-like breast cancer patients can benefit.

The final year of this study has led to the continuation of my training through collaborations and interactions with a number of clinicians, pathologists, bench scientists and epidemiologists. Additionally, I have completed cell cycle analysis studies and continued work on immunohistochemical, gene/protein expression cell cycle analysis, and *in vivo* work. Finally, preparations of dissertation and publication manuscripts have begun.

References: none

Appendices: Curriculum vitae, Era of Hope poster, AACR Advancements in Breast Cancer Research Poster

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Katerina D. Fagan-Solis, Joseph M. Gozgit, Christopher M. Otis, Sharon A. Marconi, Brian T Pentecost, Douglas L. Anderton, Sallie Smith-Schneider, Kathleen F. Arcaro. Regulation and action of Skp2 in cell and tumor models: Mechanisms underlying aggressive growth in basal-like breast cancer [abstract]. In: Proceedings of the Meeting of Advances in Breast Cancer Research: Genetics, Biology, and Clinical Applications; 2009 Oct 13-16; San Diego, CA. Philadelphia (PA): AACR; 2009.

Katerina Fagan-Solis, Joseph Gozgit, Brian Pentecost, Christopher Otis, Sharon Marconi, Sallie Smith-Schneider, Kathleen Arcaro. S-phase kinase-associated protein 2 (SKP2) in estrogen receptor-negative and triple-negative breast cancer. Vermont Cancer Center Breast Cancer Conference, October 15, 2010. Burlington, VT

Katerina D. Fagan-Solis, Christopher N. Otis, Kathleen F. Arcaro. S-phase kinase-associated protein 2 in triple-negative and basal-like breast cancer. Congressionally Directed Medical Research Programs Era of Hope 2011 Meeting; August 2-5, 2011. Orlando, FL

Katerina D. Fagan-Solis, Christopher M. Otis, Sallie W. Smith-Schneider, Kathleen F. Arcaro. S-Phase Kinase-Associated Protein 2 in Triple-Negative and Basal-Like Breast Cancer [abstract]. In: Proceedings of the Meeting of Advances in Breast Cancer Research: Genetics, Biology, and Clinical Applications; 2011 Oct 12-16; San Francisco, CA. Philadelphia (PA): AACR; 2011.

Personnel (not salaries) receiving pay from the research effort: None

Katerina D. Fagan-Solis

BUSINESS INFORMATION
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EDUCATION

2005-Present **University of Massachusetts Amherst**
Doctor of Philosophy, Molecular and Cellular Biology

2000-2004 **State University Of New York at Albany**
Bachelor of Science, Biological Chemistry and Molecular Biology
Accomplishments: Graduated Cum Laude, Spellman Award for Achievement (Spring 2001, 2002, 2003, 2004), Excelencia Latina (Spring 2003), Golden Key International Honor Society, Deans List (Fall 2000, 2001, 2002; Spring 2001, 2004)

EXPERIENCE

2005- Present
Research Assistant in the Molecular and Cellular Biology Program at UMASS Amherst
Research Project: Regulation and action of SKP2 in cell and tumor models: Mechanisms underlying aggressive growth in basal-like breast cancer
Subproject: Characterizing the invasion mechanism utilized by the TMX2-28 breast cancer cell line
Skills Acquired:
- Cell culture techniques
- Fluorescence microscopy
- Nucleotide and protein isolation and quantification
- Mammalian and bacterial cloning
- Cell cycle analysis
- Immunohistochemisty
- RNAi
- PCR (quantitative, real time, reverse transcription)
- Gel electrophoresis
- Zymography
- Tissue microarray construction
- Grant writing

Presented research projects in numerous posters and power point presentations, a number of which were at research conferences

Summer 2005
Summer Program for Undergraduate Research, UMASS Amherst
Research Project: Isolation of Adult Mammary Stem Cells from Breast Milk
Skills Acquired:
Cell culture techniques
Magnetic cell sorting
Fluorescence microscopy
RNA isolation
Presented research project at poster session

Summer 2002
Ronald E. McNair Post-Baccalaureate Achievement Program, SUNY Albany
Research Project: The Effects of Blocking Intracellular Steroid Receptors in the Hippocampus or Amygdala on Learning and Memory
Skills Acquired:
Animal husbandry
Inter- cannulae infusion, hormone inserts and injections
Determination of behavioral estrus in rats
Inhibitory avoidance testing
Presented research project at McNair Scholars' Day

TEACHING ASSISTANTSHIPS:

Introductory Biology I Laboratory (BIO 100)	Fall '06

MENTORSHIP (of incoming graduate students)

Northeast Alliance Graduate Mentorship Program	Fall '06, Fall '08
Molecular and Cellular Biology Graduate Program	Fall '08, Fall '11
Arcaro Lab	Fall '08-present

AWARDS

Carl Storm Underrepresented Minority Fellowship	August '06
SACNAS Travel Scholarship	October '06
AACR Minority Scholar in Cancer Research Award	April '07, October '11
Northeast Alliance Graduate Fellowship	Fall '06,'07,'11; Spring '06,'08, '12
Research Assistantship	'06-Present
Preparing Future Faculty Summer Institute	June '07

POSTER ABSTRACTS AND ORAL PRESENTATIONS

Katerina D. Fagan-Solis, Joseph M. Gozgit, Kathleen F. Arcaro**.** *RNA Silencing of SKP2 in the Estrogen Receptor-Negative Breast Cancer Cell Line, TMX2-28.* Cancer Models & Mechanisms Gordon Research Conference, July 30 - August 4, 2006. Smithfield, RI

Katerina D. Fagan-Solis, Joseph M. Gozgit, Kathleen F. Arcaro**.** *RNA Silencing of SKP2 in the Estrogen Receptor-Negative Breast Cancer Cell Line, TMX2-28.* Society for Advancement of

Chicanos and Native Americans in Science National Conference, October 26- October 29, 2006. Tampa, FL

Paczkowski KE, Turk CM, Gozgit JM, Smith-Schneider SW, Marconi SA, Otis CN, Crisi GM, Anderton DL, Killiman MW, **Fagan-Solis K,** Pentecost BT, Arcaro KA. MCB Retreat Poster Session. "Paralemmin, a morphoregulatory protein, is differentially expressed between normal and breast cancer tissue". 2008. Amherst, MA

Katerina Fagan-Solis, Joseph Gozgit, Brian Pentecost, Christopher Otis, Sharon Marconi, Sallie Smith-Schneider, Kathleen Arcaro. *S-phase kinase-associated protein 2 (SKP2) in estrogen receptor-negative and triple-negative breast cancer.* Vermont Cancer Center Breast Cancer Conference, October 15, 2010. Burlington, VT

Katerina D. Fagan-Solis, Christopher N. Otis, Kathleen F. Arcaro. *S-phase kinase-associated protein 2 in triple-negative and basal-like breast cancer.* Congressionally Directed Medical Research Programs Era of Hope 2011 Meeting; August 2-5, 2011. Orlando, FL

POSTER PUBLICATIONS

Katerina Fagan-Solis, Joseph Gozgit, Christopher Otis, Sharon Marconi, and Kathleen Arcaro The role of SKP2 in the proliferative and aggressive nature of estrogen receptor negative breast cancer. [abstract]. In: Proceedings of the 98th Annual Meeting of the American Association for Cancer Research; 2007 Apr 14-18; Los Angeles, CA. Philadelphia (PA): AACR; 2007. Abstract nr 5208.

Katerina D. Fagan-Solis, Brian T. Pentecost, Kathleen F**.** Arcaro. Role of mitogen-inducible gene 2 in breast cancer metastasis. [abstract]. In: Proceedings of the 99th Annual Meeting of the American Association for Cancer Research; 2008 Apr 12-16; San Diego, CA. Philadelphia (PA): AACR; 2008. Abstract nr 2884.

Katerina D. Fagan-Solis, Joseph M. Gozgit, Christopher M. Otis, Sharon A. Marconi, Brian T Pentecost, Douglas L. Anderton, Sallie Smith-Schneider, Kathleen F. Arcaro. Regulation and action of Skp2 in cell and tumor models: Mechanisms underlying aggressive growth in basal-like breast cancer [abstract]. In: Proceedings of the Meeting of Advances in Breast Cancer Research: Genetics, Biology, and Clinical Applications; 2009 Oct 13-16; San Diego, CA. Philadelphia (PA): AACR; 2009.

Katerina D. Fagan-Solis, Sallie Smith-Schneider, Kathleen F**.** Arcaro. Inhibiting the Rho pathway in the triple-negative, basal-like breast cancer cell line, TMX2-28, inhibits invasive behavior [abstract]. In: Proceedings of the 102th Annual Meeting of the American Association for Cancer Research; 2011 Apr 2-6; Orlando, FL. Philadelphia (PA): AACR; 2011. Abstract nr 1403.

Katerina D. Fagan-Solis, Christopher M. Otis, Sallie W. Smith-Schneider, Kathleen F. Arcaro. S-Phase Kinase-Associated Protein 2 in Triple-Negative and Basal-Like Breast Cancer [abstract]. In: Proceedings of the Meeting of Advances in Breast Cancer Research: Genetics,

Biology, and Clinical Applications; 2011 Oct 12-16; San Francisco, CA. Philadelphia (PA): AACR; 2011.

Kristin E. Williams**, Katerina D. Fagan-Solis**, Kathleen F. Arcaro. Estrogen receptor-α and ras homolog gene family, member A in a tamoxifen-selected cell line are not controlled by promoter methylation [abstract]. In: Proceedings of the Meeting of Advances in Breast Cancer Research: Genetics, Biology, and Clinical Applications; 2011 Oct 12-16; San Francisco, CA. Philadelphia (PA): AACR; 2011.

PUBLICATIONS

Moffatt LT, **Fagan-Solis KD**, Browne EP, Arcaro KF, 2011. Vitellogenin and 11β hydroxylase mRNA expression in male Japanese medaka (Oryzias latipes) exposed in a short term laboratory assay to low levels of three estrogenic contaminants. *International Society of Environmental Indicators.* 6(1).

Turk CM, **Fagan-Solis K**, Paczkowski KE, Gozgit JM, Smith-Schneider S, Marconi SA, Otis CN, Crisi G, Anderton DL, Kilimann MW, Arcaro KF. "Paralemmin, a morphoregulatory protein, is differentially expressed between normal and breast cancer tissue." To be submitted to BJC: In final draft form.

PROFESSIONAL DEVELOPMENT

Preparing Future Faculty Summer Institute	June 2007

PROFESSIONAL SOCIETY MEMBERSHIPS

Society for Advancement of Chicanos and Native Americans in Science	2006-present
American Association for Cancer Research	2006-present
American Association for Cancer Research: Women in Cancer Research	2006-present
American Association for Cancer Research: Minorities in Cancer Research	2006-present
American Association for the Advancement of Science	2008-present

RESEARCH GRANTS

Recent: Department of Defense Predoctoral Traineeship Award ; 2008-2011
Role: Principal Investigator (100% effort)
Recent: Rays of Hope; Baystate Medical Center ; 2009-2010
Role: Co-Principal Investigator (100% effort)
Recent: Rays of Hope; Baystate Medical Center ; 2007-2008
Role: Co-Principal Investigator (100% effort)
Recent: Rays of Hope; Baystate Medical Center ; 2006-2007
Role: Supported researcher (100% effort)
Recent: Rays of Hope; Baystate Medical Center ; 2005-2006
Role: Supported researcher (100% effort)

S-Phase Kinase-Associated Protein 2 in Triple-Negative and Basal-Like Breast Cancer

Katerina D. Fagan-Solis[1, 4], Christopher N. Otis[2], Kathleen F. Arcaro [3,4]

[1]Molecular and Cellular Biology Program, [2]Director of Surgical Pathology, Baystate Medical Center, Springfield, MA,
[3]Department of Veterinary and Animal Science, [4]University of Massachusetts, Amherst, MA

University of Massachusetts Amherst

Poster ID
P56-9

BACKGROUND

- Breast cancer is a heterogeneous disease that varies in its biology and response to therapy.
- Historically, estrogen receptor (ER) is the most important prognostic factor in breast cancer, dictating a patient's therapeutic regimen.
- Currently, breast tumors are further classified into subtypes based on their gene expression patterns.
  - Triple-negative tumors are a subset typically lacking ER, progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression.
  - Basal-like tumors are associated with positive basal cytokeratin (CK) 5, 14, and/or 17 expression patterns.
    - Patients with these tumor subsets face poor prognosis as they will not be responsive to antiestrogen nor anti-HER2 therapies.
- Designing individualized treatment for specific subgroups of disease requires targeting genes or pathways that are differentially expressed or activated.
- The objective of this research is to further our understanding of the molecular mechanisms underlying the aggressive growth associated with triple-negative and basal-like breast tumors.
- **We hypothesize that overexpression of SKP2 and subsequent dysregulation of the cell cycle plays a role in the development of the highly proliferative and aggressive nature of triple-negative and basal-like breast cancers.**
  - Through study of human tissue, cell culture, and animal models, expression patterns of SKP2 and its associated proteins in breast cancer can be determined.

SKP2 PATHWAY

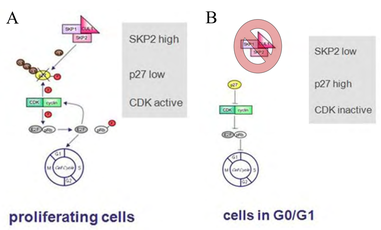

Figure 1: SKP2 promotes progression into the S-phase of the cell cycle by regulating p27. (A) In proliferating cells, SKP2 targets p27 for ubiquitin mediated degradation. By targeting p27 for degradation, SKP2 promotes progression into the S-phase of the cell cycle. (B) In the absence of SKP2, p27 abrogates the actions of cyclin/CDK complexes thereby preventing the G_1-S transition and inhibiting the cell cycle.

(Modified from: http://www.liferaftgroup.org/gist_news/index.php?option=com_zine&view=article&id=226:defining-molecular-mechanisms-of-imatinib-treatment-in-gist)

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3. Gozgit, J., Pentecost, BT, Marconi, SA, Otis, CN, Wu, C, Arcaro, KF, Use of an aggressive MCF-7 cell line variant, TMX2-28, to study cell invasion in breast cancer. *Molecular Cancer Research* 2006. 4(12): p. 905-13.
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RESULTS

STUDY OF HUMAN TISSUE

SKP2 mRNA is Overexpressed in Basal-Like Breast Tumors

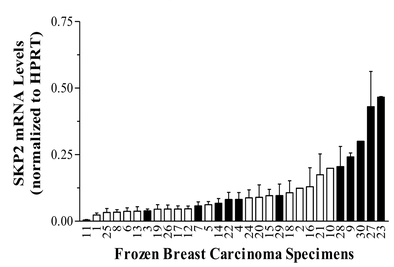
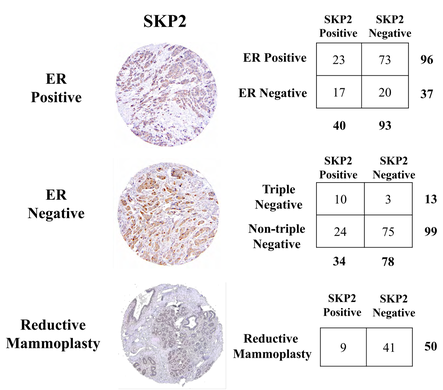


Figure 2: Gene expression of SKP2 was determined in 30 frozen breast carcinoma samples using real time qRT-PCR. Tumors were sorted by SKP2 expression. Tumors with CKs 5 and/or 17 positivity are shaded. ER-positive tumors were assigned the numbers 1-18 while ER-negative tumors were assigned 19-30.

SKP2 Protein is Highly Expressed in 46% of ER-Negative and 77% of Triple-Negative Breast Cancers



	SKP2 Positive	SKP2 Negative	
ER Positive	23	73	96
ER Negative	17	20	37
	40	93	

	SKP2 Positive	SKP2 Negative	
Triple Negative	10	3	13
Non-triple Negative	24	75	99
	34	78	

	SKP2 Positive	SKP2 Negative	
Reductive Mammoplasty	9	41	50

Figure 3: SKP2 expression was examined in 37 ER-negative, 96 ER-positive, and 50 reductive mammoplasty tissue samples by immunohistochemistry. SKP2 was highly expressed in 46% (17 of 37) of ER-negative tumors, 24% (23 of 96) of ER-positive tumors, and 18% (9 of 50) reductive mammoplasty tissues. Importantly, SKP2 was highly expressed in 77% (10 of 13) of triple-negative breast cancers while only 24% (24 of 99) of non-triple negative breast cancers had high expression of SKP2. Representative SKP2 stained punches are shown.

STUDY OF CELL CULTURE

TMX2-28 Cells are Triple-Negative and Have a Mixed Basal/Luminal Cytokeratin mRNA Expression

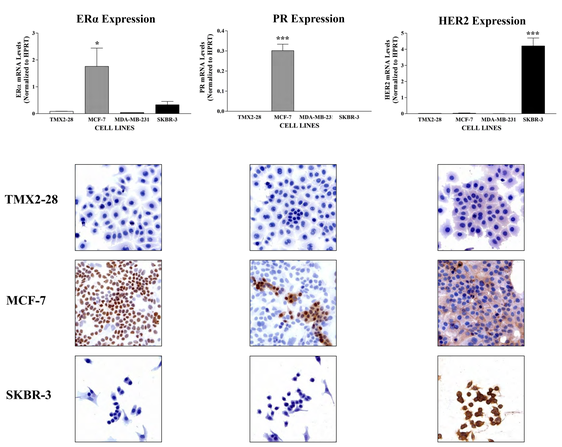


Figure 4: mRNA expression of ERα, PR, and HER2 was determined by real time qRT-PCR. Protein expression of ERα, PR, and HER2 was determined by immunohistochemistry.

Table 1: CK mRNA Expression in TMX2-28 Cells. Data are expressed as fold change in TMX2-28 compared to MCF-7 cells.

		Fold Change			Fold Change
BASAL CYTOKERATINS	CK5	22	LUMINAL CYTOKERATINS	CK8	0.98
	CK14	45		CK18	1.2
	CK17	7		CK19	-19
				CK20	-100

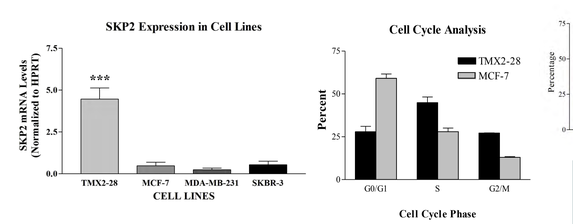


Figure 5: SKP2 mRNA expression in cell lines was determined using real time qRT-PCR. Cell cycle analysis was determined by Flow Cytometry (*FACS*) analysis.

TMX2-28 Cells Overexpress a Number of Cell Cycle Genes Associated With SKP2

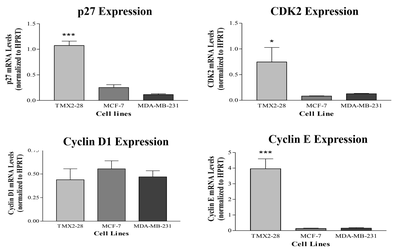


Figure 6: Gene expression was determined using real time qRT-PCR.

Table 2: mRNA and protein expression of SKP2 and its associated genes in TMX2-28 cells, 48 hours post transient knockdown of SKP2.

Gene	mRNA Expression	Protein Expression
SKP2	Decrease (~70%)	Decrease (~70%)
p27	No significant change	Currently being determined
CDK2		
CYCLIN D1		
CYCLIN E		

Knockdown of SKP2 in TMX2-28 Results in a Significant Increase in the Percentage of Cells in the G0/G1 phase and Decrease of Cells in the S-phase of the Cell Cycle

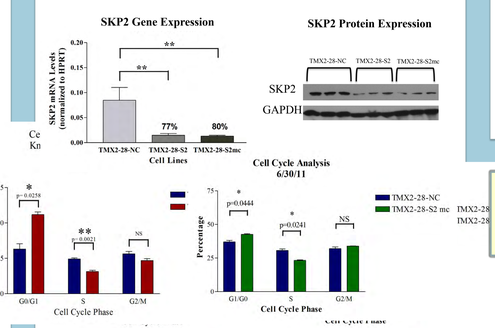


Figure 7: TMX2-28 cells were stably transfected with shRNA targeting SKP2 or negative control shRNA. RNA was isolated from both a mass (TMX2-28-S2mc) and a clonally selected (TMX2-28-S2) knockdown population. Gene expression was determined using real time qRT-PCR. Protein expression was determined by western immunoblot analysis. Cell cycle analysis was determined by Flow Cytometry (*FACS*) analysis.

STUDY OF ANIMAL MODELS

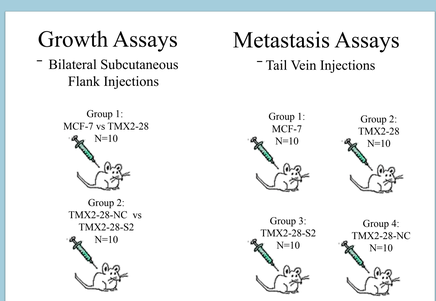


Figure 8: Schematic representation of ongoing *in vivo* studies.

CONCLUSIONS

- Numerous researchers have suggested that SKP2 may provide a good target for therapy.
- SKP2 is highly expressed in 46% of ER-negative and 77% of triple-negative breast cancers as opposed to 24% of ER-positive and 24% of non-triple negative breast cancers.
- TMX2-28 cells are a Tamoxifen-selected, MCF-7 variant that have a triple-negative and basal-like expression pattern.
- TMX2-28 cells overexpress SKP2 as well as a number of its cell cycle associated genes including p27, CDK2, and cyclin E.
- Knockdown of SKP2 in TMX2-28 cells shifted the cell cycle resulting in a significant increase in the percentage of cells in the G1/G0 phase, as well as a significant decrease in the percentage of cells in the S-phase of the cell cycle.
- Current data suggest that overexpression of SKP2 and the subsequent dysregulation of the cell cycle plays a role in the development of the highly proliferative and aggressive nature of triple-negative and basal-like breast cancers.

FUNDING

This work was supported by the U.S. Army Medical Research and Materiel Command under W81XWH-08-1-0345 and the Rays of Hope organization

Baystate Health
Rays of Hope
Congressionally Directed Medical Research Programs CDMRP Department of Defense

S-Phase Kinase-Associated Protein 2 in Triple-Negative and Basal-Like Breast Cancer

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[3]Pioneer Valley Life Sciences Institute, [4]Department of Veterinary and Animal Science, [5]University of Massachusetts, Amherst, MA

BACKGROUND

- Breast cancer is a heterogeneous disease that varies in its biology and response to therapy.
- Historically, estrogen receptor (ER) is the most important prognostic factor in breast cancer, dictating a patient's therapeutic regimen.
- Currently, breast tumors are further classified into subtypes based on their gene expression patterns.
  - Triple-negative tumors are a subset typically lacking ER, progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) expression.
  - Basal-like tumors are associated with positive basal cytokeratin (CK) 5, 14, and/or 17 expression patterns.
    - Patients with these tumor subsets face poor prognosis as they will not be responsive to antiestrogen nor anti-HER2 therapies.
- Designing individualized treatment for specific subgroups of disease requires targeting genes or pathways that are differentially expressed or activated.
- The objective of this research is to further our understanding of the molecular mechanisms underlying the aggressive growth associated with triple-negative and basal-like breast tumors.
- **We hypothesize that overexpression of SKP2 and subsequent dysregulation of the cell cycle plays a role in the development of the highly proliferative and aggressive nature of triple-negative and basal-like breast cancers.**
  - Through study of human tissue, cell culture, and animal models, expression patterns of SKP2 and its associated proteins in breast cancer can be determined.

SKP2 PATHWAY

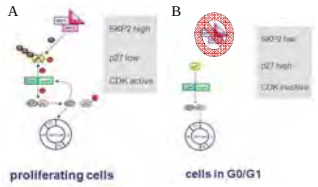

Figure 1: SKP2 promotes progression into the S-phase of the cell cycle by regulating p27. (A) In proliferating cells, SKP2 targets p27 for ubiquitin mediated degradation. By targeting p27 for degradation, SKP2 promotes progression into the S-phase of the cell cycle. (B) In the absence of SKP2, p27 abrogates the actions of cyclin/CDK complexes thereby preventing the G_1-S transition and inhibiting the cell cycle.

(Modified from: http://www.lilealtgroup.org/gist_news/index.php?option=com_zine&view=article&id=226:defining-molecular-mechanisms-of-imatinib-treatment-in-gist)

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RESULTS

STUDY OF HUMAN TISSUE

SKP2 mRNA is Overexpressed in Basal-Like Breast Tumors

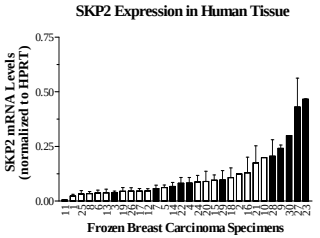
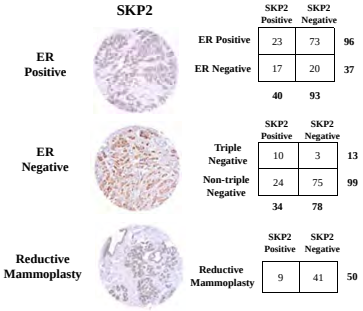


Figure 2: Gene expression of SKP2 was determined in 30 frozen breast carcinoma samples using real time qRT-PCR. Tumors were sorted by SKP2 expression. Tumors with CKs 5 and/or 17 positivity are shaded. ER-positive tumors were assigned the numbers 1-18 while ER-negative tumors were assigned 19-30.

SKP2 Protein is Highly Expressed in 46% of ER-Negative and 77% of Triple-Negative Breast Cancers



	SKP2 Positive	SKP2 Negative	
ER Positive	23	73	96
ER Negative	17	20	37
	40	93	

	SKP2 Positive	SKP2 Negative	
Triple Negative	10	3	13
Non-triple Negative	24	75	99
	34	78	

	SKP2 Positive	SKP2 Negative	
Reductive Mammoplasty	9	41	50

Figure 3: SKP2 expression was examined in 37 ER-negative, 96 ER-positive, and 50 reductive mammoplasty tissue samples by immunohistochemistry. SKP2 was highly expressed in 46% (17 of 37) of ER-negative tumors, 24% (23 of 96) of ER-positive tumors, and 18% (9 of 50) reductive mammoplasty tissues. Importantly, SKP2 was highly expressed in 77% (10 of 13) of triple-negative breast cancers while only 24% (24 of 99) of non-triple negative breast cancers had high expression of SKP2. Representative SKP2 stained punches are shown.

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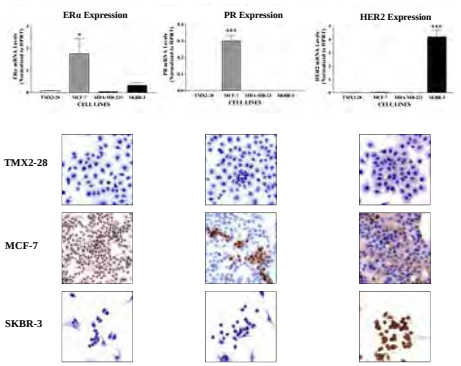

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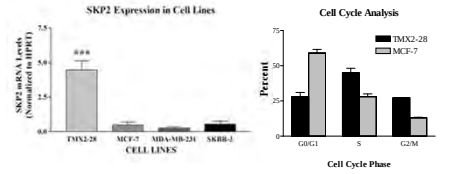

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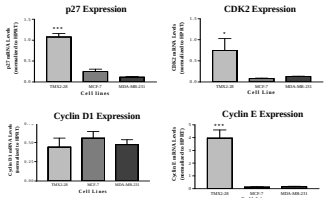

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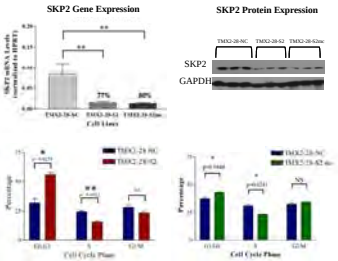

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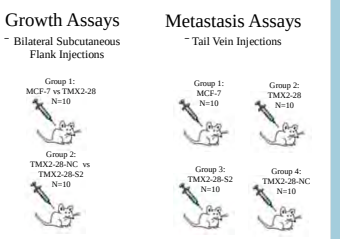

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